

AMENDMENTS

The Examiner is respectfully requested to make the following amendments.

IN THE CLAIMS:

1. (Withdrawn) A method for identifying genes having an expression pattern in tumor cells that is modulated when the cells are contacted with a chemotherapeutic agent comprising the steps of:

a) separating living neoplastic cells from dead cells, vascular endothelial cells and living stromal cells in a mixed population of cells from a tumor sample, by

 i) contacting, the mixed population of cells with a vital stain or fluorescent dye;

 ii) contacting the mixed population of cells with a detectably-labeled immunological reagent that specifically binds to neoplastic cells; and

 iii) selecting the cells in the mixed population of step (b) that are not stained with the vital stain and that bind the immunological reagent,

b) contacting the separated, living neoplastic cells with a chemotherapeutic amount of a chemotherapeutic agent;

c) separating apoptotic and non-apoptotic cells of step b) by contacting the cells with a reagent specific for apoptosis and sorting the population of cells in step b) thereby;

d) assaying gene expression in each of the separated populations of apoptotic and non-apoptotic cells; and

e) identifying genes having an expression pattern that is modulated by contacting cells with a chemotherapeutic amount of a chemotherapeutic agent.

2. (Withdrawn) A method according to claim 1, wherein expression of one or a plurality of genes is increased in cells sensitive to the chemotherapeutic agent.

3. (Withdrawn) A method according to claim 1, wherein expression of one or a plurality of genes is increased in cells resistant to the chemotherapeutic agent.

4. (Withdrawn) A gene whose expression is increased according to the method of

claim 2, wherein the gene is Sjogren syndrome antigen B (autoantigen La), capping protein alpha, adenine nucleotide translocator 3 (liver), AU-rich element RNA-binding protein AUF1, phosphatidylserine synthase I, integrin, alpha L, lymphocyte function-associated antigen 1, branched chain keto acid dehydrogenase E1, alpha polypeptide, annexin XI (56 kD autoantigen), or Von Hippel-Lindau syndrome.

5. (Withdrawn) A gene whose expression is increased according to the method of claim 3, wherein the gene is myosin phosphatase target subunit 1 (MYPT1), albumin D-box binding protein, complement component 7, plasminogen activator, urokinase receptor, ATPase, DNA binding protein (HIP 116), zinc finger protein (ZNF198) or tropomodulin.

6. (Withdrawn) A method for identifying one or a plurality of genes having a pattern of expression that is different in a tumor cell sensitive to a chemotherapeutic drug than the expression pattern in a tumor cell resistant to the chemotherapeutic drug, the method comprising the steps of:

a) performing an extreme drug resistance (EDR) assay on a mixed population of cells from a tumor sample;

b) separating living tumor cells from dead cells, vascular endothelial cells and living stromal cells in a mixed population of cells from a tumor sample, by

i) contacting the mixed population of cells with a vital stain or fluorescent dye;

ii) contacting the mixed population of cells with a detectably-labeled immunological reagent that specifically binds to neoplastic cells; and

iii) selecting the cells in the mixed population that are not stained with the vital stain and that bind the immunological reagent,

c) assaying gene expression in each of the separated populations of drug sensitive and drug resistant cells; and

d) identifying genes having an expression pattern that is different in the drug resistant cells than in the drug sensitive cells.

7. (Withdrawn) A method according to claim 6, wherein expression of one or a

plurality of genes is increased in cells resistant to the chemotherapeutic agent.

8. (Withdrawn) A method according to claim 6, wherein expression of one or a plurality of genes is increased in cells sensitive to the chemotherapeutic agent.

9. (Withdrawn) A method according to claim 1, wherein expression of one or a plurality of genes is decreased in cells resistant to the chemotherapeutic agent.

10. (Withdrawn) A method according to claim 1, wherein expression of one or a plurality of genes is decreased in cells sensitive to the chemotherapeutic agent.

11. (Withdrawn) A gene whose expression is increased according to the method of claim 8, wherein the gene is Sjogren syndrome antigen B (autoantigen La), capping protein alpha, adenine nucleotide translocator 3 (liver), AU-rich element RNA-binding protein AUF1, phosphatidylserine synthase I, integrin, alpha L, lymphocyte function-associated antigen 1, branched chain keto acid dehydrogenase E1, alpha polypeptide, annexin XI (56 kD autoantigen), or Von Hippel-Lindau syndrome.

12. (Withdrawn) A gene whose expression is increased according to the method of claim 7, wherein the gene is myosin phosphatase target subunit 1 (MYPT1), albumin D-box binding protein, complement component 7, plasminogen activator, urokinase receptor, ATPase, DNA binding protein (HIP 116), zinc finger protein (ZNF198) or tropomodulin.

13. (Withdrawn) The method of claim 1, wherein the cell is a breast cancer tumor cell.

14. (Withdrawn) The method of claim 6, wherein the cell is a breast cancer tumor cell.

15. (Withdrawn) The method of claim 1, wherein the chemotherapeutic drug is a taxane.

16. (Withdrawn) The method of claim 6, wherein the chemotherapeutic drug is a taxane.

17. (Withdrawn) The method of claim 15, wherein the chemotherapeutic drug is a taxane, docetaxel or paclitaxel.

18. (Withdrawn) A pattern of gene differential gene expression, comprising increased expression of one or a plurality of genes that are myosin phosphatase target subunit 1 (MYPT1), albumin D-box binding protein, complement component 7, plasminogen activator, urokinase receptor, ATPase, DNA binding protein (HIP 116), zinc finger protein (ZNF198) or tropomodulin, wherein increased expression of said one or plurality of genes in a tumor cell compared with a non-tumor cell identifies said tumor cell to be a cell resistant to chemotherapeutic drugs that are taxanes.

19. (Withdrawn) A pattern of gene differential gene expression, comprising increased expression of one or a plurality of genes that are Sjogren syndrome antigen B (autoantigen La), capping protein alpha, adenine nucleotide translocator 3 (liver), AU-rich element RNA-binding protein AUF1, phosphatidylserine synthase I, integrin, alpha L, lymphocyte function-associated antigen 1, branched chain keto acid dehydrogenase E1, alpha polypeptide, annexin XI (56 kD autoantigen), or Von Hippel-Lindau syndrome wherein increased expression of said one or plurality of genes in a tumor cell compared with a non-tumor cell identifies said tumor cell to be a cell sensitive to chemotherapeutic drugs that are taxanes.

20. (Original) A method for identifying a tumor or cells comprising the tumor that are resistant to taxane chemotherapeutic drugs, the method comprising the steps of:

a) determining gene expression levels in a tumor sample or cells comprising the tumor for one or a plurality of genes that are myosin phosphatase target subunit 1 (MYPT1), albumin D-box binding protein, complement component 7, plasminogen activator, urokinase receptor, ATPase, DNA binding protein (HIP 116), zinc finger protein (ZNF198) or tropomodulin;

b) comparing gene expression levels of the one or plurality of genes in step a) with gene expression levels of said one or plurality of genes in a non-tumor sample or cells comprising said sample; and

c) identifying the tumor or cells comprising the tumor to be resistant to taxane

chemotherapeutic drugs when the gene expression levels of one or a plurality of said genes is increased in the tumor sample when compared to gene expression levels in the non-tumor sample.

21. (Original) The method of claim 20, wherein the tumor sample is a breast cancer tumor sample.

22. (Original) A method for identifying a tumor or cells comprising the tumor that are sensitive to taxane chemotherapeutic drugs, the method comprising the steps of:

a) determining gene expression levels in a tumor sample or cells comprising the tumor for one or a plurality of genes that are Sjogren syndrome antigen B (autoantigen La), capping protein alpha, adenine nucleotide translocator 3 (liver), AU-rich element RNA-binding protein AUF1, phosphatidylserine synthase I, integrin, alpha L, lymphocyte function-associated antigen 1, branched chain keto acid dehydrogenase E1, alpha polypeptide, annexin XI (56 kD autoantigen), or Von Hippel-Lindau syndrome;

b) comparing gene expression levels of the one or plurality of genes in step a) with gene expression levels of said one or plurality of genes in a non-tumor sample or cells comprising said sample; and

c) identifying the tumor or cells comprising the tumor to be sensitive to taxane chemotherapeutic drugs when the gene expression levels of one or a plurality of said genes is increased in the tumor sample when compared to gene expression levels in the non-tumor sample.

23. (Original) The method of claim 21, wherein the tumor sample is a breast cancer tumor sample.

24. (Withdrawn) The method of claim 1, wherein the vital stain is propidium iodide.

25. (Withdrawn) The method of claim 1, wherein the immunological reagent is detectably labeled with a fluorescent label.

26. (Withdrawn) The method of claim 1, wherein the immunological reagent is a

tumor-specific antibody.

27. (Withdrawn) The method of claim 1, wherein the cells of step (c) are selected by fluorescence-activated cell sorting.

28. (Withdrawn) The method of claim 1, wherein the tumor sample is a solid tumor sample and the mixed cell population is a disaggregated tumor sample.

29. (Withdrawn) The method of claim 26, wherein the tumor-specific antibody is immunologically specific for EGFR or HER2.

30. (Withdrawn) A method for detecting a gene expression profile of living neoplastic cells that are resistant to a taxane cytotoxic compound and distinguishing said profile from the gene expression profile of living neoplastic cells that are sensitive to the taxane cytotoxic compound in a mixed population of cells from a tumor sample, the method comprising the steps of:

a) contacting the mixed population of cells with the taxane cytotoxic compound for a time and at a concentration wherein the neoplastic cells that are sensitive to the taxane cytotoxic compound undergo apoptosis;

b) contacting the mixed population of step (a) with a vital stain or fluorescent dye;

c) contacting the mixed population of cells of step (b) with a discrimination compound that specifically binds to apoptotic cells;

d) contacting the mixed cell population of step (c) with a detectably-labeled immunological reagent that specifically binds to the apoptotic cell discrimination compound; and

e) separating the cells in the mixed population of step (d) that are not stained with the vital stain from the cells that are stained with the vital stain;

f) separating the cells in the mixed population of step (e) that are not stained with the vital stain and that do not bind the immunological reagent from the cells in the mixed population of step (c) that are not stained with the vital stain and that do bind the immunological reagent;

g) isolating cellular RNA from the each of the separated cells selected in step (f);

h) preparing detectably-labeled cDNA from the cellular RNA isolated in step (g);

i) hybridizing each of the cDNA preparations prepared in step (h) to a gene array comprising at least 4000 eukaryotic genes;

j) detecting a pattern of gene expression for hybridization of each of the cDNA preparations prepared from the mRNA isolated from the cells selected in step (f); and

k) comparing the pattern of gene expression detected in step (j) from hybridization of the microarray with cDNA from cells that are not stained with the vital stain and that do not bind the immunological reagent with a pattern of gene expression obtained by hybridizing cDNA prepared from cells that are not stained with the vital stain and that do bind the immunological reagent.

31. (Withdrawn) The method of claim 30, wherein the vital stain is propidium iodide.

32. (Withdrawn) The method of claim 30, wherein the discrimination compound is Annexin V.

33. (Withdrawn) The method of claim 32, wherein the immunological reagent specifically binds to Annexin V and is detectably labeled with a fluorescent label.

34. (Withdrawn) The method of claim 30, wherein the cells of step (f) are selected by fluorescence-activated cell sorting.

35. (Withdrawn) The method of claim 30, wherein the taxane cytotoxic compound is paclitaxol, taxol or docetaxol.

36. (Withdrawn) The method of claim 30, wherein the cDNA is detectably labeled with a fluorescent label.

37. (Withdrawn) The method of claim 30, wherein the mixed population is contacted with the cytotoxic compound under in vitro cell culture conditions whereby the cells cannot attach to a solid substrate.

38. (Withdrawn) The method of claim 30, wherein the tumor sample is a solid tumor sample and the mixed cell population is a disaggregated tumor sample.

39. (Withdrawn) The method of claim 38, wherein the tumor sample is a breast cancer tumor sample.